PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2177232/VPA/sjp	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. PCT/AU2003/000919	International Filing Date *(day/month/year)* 17 July 2003	Priority Date *(day/month/year)* 17 July 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl. [7] C12N 15/82, A01H 1/00, C12N 15/40

Applicant

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1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

[X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 8 sheet(s).

3. This report contains indications relating to the following items:

- I [X] Basis of the report
- II [ ] Priority
- III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [X] Certain observations on the international application

Date of submission of the demand 17 February 2004	Date of completion of the report 28 October 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **GARETH COOK** Telephone No. (02) 6283 2541

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000919

Basis of the report

With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-57,** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **58-65,** received on **20 October 2004** with the letter of **20 October 2004**
- [X] the drawings, pages **1-9** , as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the sequence listing part of the description:
  pages **1-27,** as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:
   - [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
   - [ ] the language of publication of the international application (under Rule 48.3(b)).
   - [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:
   - [ ] contained in the international application in written form.
   - [X] filed together with the international application in computer readable form.
   - [ ] furnished subsequently to this Authority in written form.
   - [ ] furnished subsequently to this Authority in computer readable form.
   - [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - [X] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be nonobvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos: **69**

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. **69**

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	**8, 9, 16, 20, 21**	**YES**
	Claims	**1-7, 10-15, 17-19, 22-68**	**NO**
Inventive step (IS)	Claims	**8, 9, 16, 21, 22**	**YES**
	Claims	**1-7, 10-15, 17-19, 22-68**	**NO**
Industrial applicability (IA)	Claims	**1-68**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 WO 1999/043836 A (Plant Bioscience Limited)
D2 Geijskes RJ *et al*, GenBank Accession AJ277091
D3 Hagen LS *et al*, GenBank Accession L14546
D4 Hagen LS *et al*, GenPept Accession AAA03169
D5 Huang Q *et al*, GenPept Accession AAL18494
D6 Bouhida M *et al*, GenPept Accession AAA47454

Novelty (N) and Inventive Step (IS) claims 1-68

D1, D2 and D3 each disclose the genomes and opening reading frames of Badnaviruses. Each have in ORF3 a segment of at least 40 nucleotides showing an identity of about 90% to segment in ORF3 of the invention. This is sufficient identity for a probe derived from this part of ORF3 to hybridise to the sequences of the citations under high stringency conditions. D1, D2 and D3 therefore each disclose a DNA molecule comprising a promoter located of a transcribable DNA sequence that will hybridise to a nucleic acid probe derived from the nucleotide sequence of SEQ ID NO: 1 under high stringency conditions. D1 renders claims 1 to 7, 10, 11 and 18 to 65 not novel. D2 and D3 render claims 1 to 7, 10 and 11 not novel, and render claims 18, 19 and 22 to 65 are not inventive, the claims involving routine and obvious matters in the art.

D1 shows 80.9% identity in 94 nucleotide segment at about 5092 to 5187. Claims 12 to 15 are not novel when compared to D1. Claim 15 which is appended to claim 12 only requires the nucleotide segment to be at least 18 nucleotides in length. Both D2 and D3 contain segments of at least 18 nucleotides in length with over 80% identity to SEQ ID NO: 1.

Claims 66 to 68 are methods involving use of polypeptides or polynucleotides comprising "at least a portion" of SEQ ID NO: 1, 2, 3, 4 or 5 without placing any limitation on the minimum size of the portion. D1, D2 and D3 each disclose polypeptides and polynucleotides comprising at least a portion of the sequences of the invention. Claims 66 to 68 are not novel over D1 and are not inventive over D2 or D3.

D4, D5 and D6 have been raised with respect to SEQ ID NO's: 3, 4 and 5 respectively. D5 renders part (ii) of claim 17 not novel. D6 renders parts (iii) and (iv) of claims 17 not novel.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 12 to 15 in combination are not clear. Claim 12 requires the portion of the polynucleotide to be at least 90 nucleotides in length, while claim 15 which is appended to claim 12 only requires this portion to be 18 nucleotides in length. Claim 13 requires there to be at least 85% identity, while claim 14 which is appended to claim 13 only requires 80% identity.

Claim 17 in parts (v) and (vi) refers to the amino acid sequence of SEQ ID NO: 6, however this sequence is a nucleotide sequence.

Claim 69 is not fully supported by the specification. The claim is to a method involving agents that reduce the level or functional activity of polypeptides or polynucleotides of the invention, but is not limited to only agents that directly interact with the sequences of the invention. The claim includes within its scope the use of agents that interact with, eg bind to, polypeptides or polynucleotides unrelated to the invention and as a downstream effect the expression or activity of the polypeptides and polynucleotides of the invention are modulated. The specification is considered not to support methods involving agents which do not directly interact with the polynucleotides or polypeptides of the invention. The skilled addressee could only readily identify agents which directly interact with the polypeptides or polynucleotides of the invention, and use those in the methods.

WHAT IS CLAIMED IS:

1. An isolated DNA molecule comprising a promoter or biologically active fragment thereof or variant of these, wherein the promoter is located upstream of a transcribable DNA sequence that hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions.

2. The DNA molecule of claim 1, wherein the transcribable DNA sequence is obtained from a virus.

3. The DNA molecule of claim 1, wherein the transcribable DNA sequence is obtained from a badnavirus.

4. The DNA molecule of claim 2 or claim 3, wherein the transcribable DNA sequence is expressed constitutively in a monocotyledonous plant.

5. The DNA molecule of claim 2 or claim 3, wherein the transcribable DNA sequence is expressed constitutively in a non-graminaceous monocotyledonous plant.

6. The DNA molecule of claim 5, wherein the non-graminaceous monocotyledonous plant is selected from the group consisting of *Musaceae*, taro, ginger, onions, garlic, pineapple, bromeliaeds, palms, orchids, lilies and irises.

7. The DNA molecule of claim 5, wherein the non-graminaceous monocotyledonous plant is taro.

8. The DNA molecule of claim 1, wherein the promoter comprises the sequence set forth in SEQ ID NO:6.

9. The DNA molecule of claim 8, wherein the biologically active fragment is selected from the group consisting of SEQ ID NO:7, 8 and 9.

10. The DNA molecule of claim 8, wherein the variant has at least 30% sequence identity to a sequence selected from the group consisting of SEQ ID NO:6, 7, 8 and 9.

11. The DNA molecule of claim 8, wherein the variant is capable of hybridising to a sequence selected from the group consisting of SEQ ID NO: 6, 7, 8 and 9 under at least low stringency conditions.

12. An isolated polynucleotide comprising a nucleotide sequence that corresponds or is complementary to at least a portion of the sequence set forth in SEQ ID NO:1 or to a variant thereof.

13. The polynucleotide of claim 12, wherein the variant displays at least 30% sequence identity to at least a portion of the sequence set forth in SEQ ID NO:1, which is at least 18 nucleotides in length.

14. The polynucleotide of claim 13, wherein the variant displays at least 30% sequence identity to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:3, 4 and 5.

15. The polynucleotide of claim 12, wherein the variant hybridises to at least a portion of the sequence set forth in SEQ ID NO:1, which is at least 18 nucleotides in length, under at least low stringency conditions.

16. The polynucleotide of claim 15, wherein the variant hybridises to a nucleotide sequence that encodes an amino acid sequence selected from the group consisting of SEQ ID NO:3, 4 and 5 under at least low stringency conditions.

17. An isolated polypeptide comprising an amino acid sequence that corresponds to at least a portion of the sequence set forth in any of SEQ ID NO:3, 4 or 5 or of a variant that displays at least 30% sequence identity to that sequence, wherein the portion is at least 6 amino acid residues in length.

18. A chimeric DNA construct comprising an isolated promoter or biologically active fragment thereof or variant of these, wherein the promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions, wherein the promoter or biologically active fragment or variant is operably linked to a foreign or endogenous DNA sequence to be transcribed.

19. The construct of claim 18, further comprising a 3' non-translated sequence that is operably linked to the foreign or endogenous DNA sequence and that functions in plant cells to terminate transcription and/or to cause addition of a polyadenylated nucleotide sequence to the 3' end of a transcribed RNA sequence.

20. The construct of claim 18, wherein the promoter comprises the sequence set forth in SEQ ID NO:6.

21. The construct of claim 18, wherein the biologically active fragment is selected from the group consisting of SEQ ID NO:7, 8 and 9.

22. The construct of claim 18, wherein the variant has at least 30% sequence identity to a sequence selected from the group consisting of SEQ ID NO:6,7, 8 and 9.

23. The construct of claim 18, wherein the variant is capable of hybridising to a sequence selected from the group consisting of SEQ ID NO: 6,7, 8 and 9 under at least low stringency conditions.

24. The construct of claim 18, wherein the foreign or endogenous DNA sequence encodes a structural or regulatory protein.

25. The construct of claim 18, wherein the foreign or endogenous DNA sequence encodes a transcript capable of modulating expression of a corresponding target gene.

26. The construct of claim 25, wherein the transcript comprises a transcribed region aimed at downregulating the expression of the corresponding target gene.

27. The construct of claim 25, wherein the transcript comprises a transcribed region that represents a molecule selected from the group consisting of a sense suppression molecule, an antisense RNA, a ribozyme and an RNAi molecule.

28. The construct of claim 18, further comprising an enhancer element.

29. The construct of claim 18, further comprising a leader sequence which modulates mRNA stability.

30. The construct of claim 18, further comprising a targeting sequence for targeting a protein product of the foreign or endogenous DNA sequence to an intracellular compartment within plant cells or to an extracellular environment.

31. The construct of claim 18, further comprising a selectable marker gene.

32. The construct of claim 18, further comprising a screenable marker gene.

33. The construct of claim 18, wherein the promoter or biologically active fragment or variant is constitutively expressed in a host cell.

34. The construct of claim 33, wherein the host cell is a plant cell.

35. The construct of claim 33, wherein the host cell is a monocotyledonous plant cell.

36. The construct of claim 33, wherein the host cell is a non-graminaceous monocotyledonous plant cell.

37. The construct of claim 33, wherein the host cell is a non-graminaceous monocotyledonous plant cell selected from the group consisting of *Musaceae*, taro, ginger, onions, garlic, pineapple, bromeliaeds, palms, orchids, lilies and irises.

38. The construct of claim 33, wherein the host cell is a graminaceous monocotyledonous plant cell.

39. The construct of claim 33, wherein the host cell is a dicotyledonous plant cell.

40. A method for gene expression in a plant, comprising introducing into a plant cell a chimeric DNA construct comprising an isolated promoter or biologically active fragment thereof or variant of these, wherein the promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions, wherein the promoter or biologically active fragment or variant is operably linked to a foreign or endogenous DNA sequence to be transcribed

41. A method for producing transformed plant cells, comprising:

(a) introducing into regenerable plant cells a chimeric DNA construct comprising an isolated promoter or biologically active fragment thereof or variant of these, wherein the promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions, wherein the promoter or biologically active fragment or variant is operably linked to a foreign or endogenous DNA sequence to be transcribed, so as to yield transformed plant cells; and

(b) identifying or selecting transformed plant cells.

42. A method for selecting stable genetic transformants from transformed plant cells comprising:

(a) introducing into regenerable plant cells a chimeric DNA construct comprising an isolated promoter or biologically active fragment thereof or variant of these, wherein the promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions, wherein the promoter or biologically active fragment or variant is operably linked to a foreign or endogenous DNA sequence to be transcribed, so as to yield transformed plant cells; and

(b) identifying or selecting a transformed plant cell line from said transformed plant cells.

43. A method for producing a differentiated transgenic plant, comprising:

(a) introducing into regenerable plant cells a chimeric DNA construct comprising an isolated promoter or biologically active fragment thereof or variant of these, wherein the promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions, wherein the promoter or biologically active fragment or variant

is operably linked to a foreign or endogenous DNA sequence to be transcribed, so as to yield regenerable transformed plant cells;

(b) identifying or selecting a population of transformed plant cells; and

(c) regenerating a differentiated transgenic plant from the population.

44. The method of any one of claims 40 to 43, wherein the cells are dicotyledonous plant cells.

45. The method of any one of claims 40 to 43, wherein the cells are monocotyledonous plant cells.

46. The method of any one of claims 40 to 43, wherein the cells are graminaceous monocotyledonous plant cells.

47. The method of any one of claims 40 to 43, wherein the cells are non-graminaceous monocotyledonous plant cells.

48. The method of any one of claims 40 to 43, wherein expression of the chimeric DNA construct in the transformed cells imparts a phenotypic characteristic to the transformed cells.

49. The method of any one of claim 40 to 43, wherein the construct comprises a selectable marker gene.

50. The method of any one of claim 40 to 43, wherein the construct comprises a screenable marker gene.

51. The method of claim 43, wherein expression of the chimeric DNA construct renders the differentiated transgenic plant identifiable over the corresponding non-transgenic plant.

52. The method of claim 43, further comprising obtaining progeny from the differentiated transgenic plant.

53. Progeny obtained by the method of claim 52.

54. A plant part of the differentiated transgenic plant obtained from the method of claim 43, wherein the plant part contains the chimeric construct.

55. A differentiated transgenic plant regenerated from transformed plant cells obtained by the method of claim 41.

56. A transformed plant cell containing a chimeric DNA construct comprising an isolated plant promoter or biologically active fragment thereof or variant of these, wherein said promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low

stringency conditions, wherein said promoter or biologically active fragment or variant is operably linked to a foreign or endogenous DNA sequence to be transcribed.

57. A differentiated transgenic plant comprising plant cells containing a chimeric DNA construct comprising an isolated plant promoter or biologically active fragment thereof or variant of these, wherein said promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions, wherein said promoter or biologically active fragment or variant is operably linked to a foreign or endogenous DNA sequence to be transcribed.

58. The transgenic plant of claim 57, wherein the plant is a dicotyledonous plant.

59. The transgenic plant of claim 57, wherein the plant is a monocotyledonous plant.

60. The transgenic plant of claim 57, wherein the plant is a graminaceous monocotyledonous plant.

61. The transgenic plant of claim 57, wherein the plant is a non-graminaceous monocotyledonous plant.

62. The transgenic plant of claim 57, wherein the construct comprises a selectable marker gene.

63. The transgenic plant of claim 57, wherein the construct comprises a screenable marker gene.

64. The transgenic plant of claim 57, wherein the expression of the chimeric DNA construct renders the differentiated transgenic plant identifiable over the corresponding non-transgenic plant.

65. Use of a chimeric DNA construct comprising an isolated plant promoter or biologically active fragment thereof or variant of these, wherein said promoter is naturally located upstream of a transcribable DNA sequence which hybridises to a nucleic acid probe derived from the polynucleotide sequence set forth in SEQ ID NO:1 under at least low stringency conditions, wherein said promoter or biologically active fragment or variant is operably linked to a foreign or endogenous DNA sequence to be transcribed, in the production of a transformed plant cell, plant or plant part.

66. A method for diagnosing a badnaviral infection of a plant, comprising detecting the presence in a cell or tissue of the plant of (a) a nucleotide sequence that corresponds or is complementary to at least a portion of the nucleotide sequence set forth in SEQ ID NO:1 or 2, or of a variant of the nucleotide sequence, or (b) an amino acid sequence that corresponds to at least a portion of the sequence set forth in SEQ ID NO:3, 4 or 5, or of a variant of the amino acid sequence.

67. A method of screening for an agent that modulates badnaviral infection, the method comprising:

– contacting a preparation comprising:

(i) a polypeptide comprising an amino acid sequence that corresponds to at least a portion of the sequence set forth in SEQ ID NO: 3, 4 or 5, or of a variant of the sequence; or

(ii) a polynucleotide comprising a nucleotide sequence that corresponds or is complementary to at least a portion of the sequence set forth in SEQ ID NO:1 or 2, which polynucleotide is operably linked to a promoter; or

(iii) a polynucleotide comprising a reporter gene that is operably connected to a promoter comprising the sequence set forth in SEQ ID NO:6, 7, 8 or 9,

with a test agent; and

– detecting a change in the level and/or functional activity of the polypeptide, or an expression product of the nucleotide sequence or of the reporter gene, relative to a normal or reference level and/or functional activity in the absence of the test agent.

68. The method of claim 67, wherein the agent inhibits or reduces badnavirus infection and the method comprises detecting a reduction in the level and/or functional activity of the polypeptide, or an expression product of the nucleotide sequence or of the reporter gene, relative to the normal or reference level and/or functional activity.

69. A method for treating and/or preventing badnaviral infection of a plant, comprising administering to the plant an agent that:

– reduces the level and/or functional activity of:

a polypeptide that comprises an amino acid sequence corresponding to at least a portion of the sequence set forth in SEQ ID NO: 3, 4 or 5, or of a variant of the sequence; or

an expression product of a nucleotide sequence that corresponds or is complementary to at least a portion of the sequence set forth in SEQ ID NO:1 or 2; or

– reduces the functional activity of a promoter that comprises the sequence set forth in any one of SEQ ID NO:6, 7, 8 or 9.